Received: 29 November 2020 | Revised: 22 April 2021 | Accepted: 28 April 2021

DOI: 10.1111/ejss.13124

ORIGINAL ARTICLE

European Journal of Soil Science WILEY

Emissions of nitrous oxide, dinitrogen and carbon dioxide from three soils amended with carbon substrates under varying soil matric potentials

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Funding information
China Scholarship Council, Grant/Award Number: 201606180104; New Zealand Agricultural Greenhouse Gas Research Centre

Abstract

Carbon (C) substrates are critical for regulating denitrification, a process that results in nitrous oxide (N_2O) and dinitrogen (N_2) emissions from soil. However, the impacts of C substrates on concomitant soil emissions of carbon dioxide (CO_2) and N_2O under varying soil types and soil water contents are not well studied. Three repacked Pallic grassland soils, varying in texture and phosphorus (P) status, containing NO_3^--^{15}N were held at three levels of matric potential (ψ, −3, −5 and −7 kPa), while receiving daily substrate additions (acetate, glucose and water control) for 14 days. The CO_2 and N_2O emissions were measured daily. Additionally, the N_2O:($N_2 + N_2O$) ratios were determined using ^{15}N on days 3 and 14. Results showed that N_2O emissions increased exponentially as soil gas diffusivity declined, and N_2O peak emissions were higher with glucose than with acetate addition, with a range (± standard deviation) of 0.1 ± 0.0 to 42.7 ± 2.1 mg N m^{-2} h^{-1}. The highest cumulative N_2O emission (2.5 ± 0.2 g N m^{-2}) was measured following glucose addition with a soil ψ of −3 kPa. In comparison with added glucose, acetate resulted in a twofold increase in N_2 emissions in soils with relatively low gas diffusivities. The N_2O:($N_2O + N_2$) emissions ratios varied with substrate (glucose, 0.91; acetate, 0.81) on day 3, and had declined by day 14 under substrate addition (≤0.10). Cumulative CO_2 emissions were enhanced with increasing soil gas diffusivity and were higher for soils amended with glucose (ranging from 22.5 ± 1.3 to 36.6 ± 1.8, g C m^{-2}) than for those amended with acetate. Collectively, the results demonstrate that the increase of N_2O, N_2 and CO_2 emissions and changes in the N_2O:($N_2 + N_2O$) ratio vary over time in response to C substrate type and soil gas diffusivity.

Highlights

- Co-regulation of CO_2 and N_2O emissions was assessed for varying soil types and C substrates.
- Soil diffusivity explained concurrently cumulative N_2O and CO_2 emissions.
- Acetate enhanced N_2O reduction to N_2 in three grassland soils more than glucose.

- C substrate effects on soil N_2O, N_2 and CO_2 emissions were soil type specific.

KEYWORDS
acetate, glucose, greenhouse gas emissions, matric potential, soil diffusivity

1 | INTRODUCTION

Nitrous oxide (N_2O) is a potent greenhouse gas and N_2O emissions from agricultural sources and synthetic fertilizers account for 6% of total anthropogenic radiative forcing (Davidson, 2009). In agricultural soils, N_2O is produced from nitrification under aerobic conditions by ammonia oxidizing bacteria (AOB) and archaea (AOA) that ultimately convert ammonia, via nitrite (NO_2^-), to nitrate (NO_3^-) (Firestone & Davidson, 1989). During hypoxic conditions AOB switch to "nitrifier denitrification", producing N_2O via NO_2^- reduction, whereas under anaerobic conditions, AOB may also produce N_2O via the anaerobic oxidation of hydroxylamine (Stein, 2019). In addition, the nitrification intermediaries (hydroxylamine, nitric oxide (NO) and NO_2^-) may undergo abiotic or biotic processes to produce N_2O (Stein, 2019). Under anaerobic conditions, denitrification sequentially reduces NO_3^- to the environmentally benign dinitrogen (N_2), with N_2O an obligate intermediary (Zumft, 1997). Hence, both the microbial production of N_2O, and its reduction to N_2 depend on the soil's oxygen (O_2) status, which is affected by the interaction between O_2 supply and consumption.

Soil pores may be filled with water or gas and so a soil's O_2 status is strongly influenced by water content, such that increasing soil water content results in a decreasing soil gas volume. Consequently, soil water-filled pore space (WFPS) has long been regarded as a measure of the potential for a soil to denitrify (e.g., Linn & Doran, 1984). However, Farquharson and Baldock (2007) cautioned against the use of WFPS to predict N_2O emissions, as the relationship varies with soil bulk density. This was demonstrated clearly by Balaine et al. (2013), who showed that peak emissions of N_2O did not occur at constant values of WFPS when soil varied across a range of bulk densities and matric potentials. The relationship was best described as a function of the soil's relative gas diffusivity (D_p/D_o, where D_p is the gas diffusion coefficient in the soil and D_o is the gas diffusion coefficient of the same gas in free air). Nitrous oxide reductase is also sensitive to the soil O_2 concentration and Balaine, Clough, Beare, Thomas, and Meenken (2016) went on to show that the ratio of N_2O:N_2 could also be explained by changes in soil D_p/D_o. This variable is indicative of the soil's O_2 supply.

Most denitrifiers are aerobic heterotrophs that use a carbon (C) source as an electron donor to reduce N oxides under anaerobic conditions (Zumft, 1997). The quantity and quality of soil C can also affect the rate of denitrification and the N_2O:N_2 ratio (Firestone & Davidson, 1989; Gillam, Zebarth, & Burton, 2008; Senbayram, Chen, Budai, Bakken, & Dittert, 2012). As the quantity of C available to denitrifiers increases, the rate of denitrification increases if sufficient NO_3^- substrate and anaerobic conditions are present (Senbayram et al., 2012). In pasture soils, C substrates are derived from a wide range of sources that include the mineralization of soil organic matter, plant root exudation, and the deposition of manures and slurries (Henry et al., 2008; Laughlin & Stevens, 2002). Increasing availability of labile C in the soil can enhance O_2 consumption due to increased respiration, potentially enhancing anaerobic conditions that favour denitrification and the production of N_2O (Friedl et al., 2018; Petersen, Ambus, Elsgaard, Schjønning, & Olesen, 2013).

Dual regulation of N_2O emissions due to variations in C form and O_2 concentration has been demonstrated: addition of butyrate and glutamic acid to soil slurries caused greater N_2O production relative to glucose and mannitol after 110 h in the presence of NO_3^- at 21% O_2 but not at ~2% O_2 (Morley & Baggs, 2010). The higher N_2O production at 21% O_2 was thought to result from the increased availability of labile C lowering O_2 concentrations (Morley & Baggs, 2010) but this was not tested. Previously, it was also shown that the efficiency of N_2O reduction to N_2 was substrate dependent and it was proposed that this effect may vary with soil type (Morley, Richardson, & Baggs, 2014; Paul, Beauchamp, & Trevors, 1989).

Furthermore, the potential for an enhanced C substrate supply to increase soil respiration and thus modify O_2 supply (soil D_p/D_o) has not been investigated with respect to N_2O and/or N_2 production under controlled conditions. Thus, the objective of this study was to vary both soil matric potential, in order to modify O_2 supply (D_p/D_o), and C substrate supply, in order to modify O_2 consumption (increased respiration), and to determine their combined effects on the production of N_2O and N_2. For a given soil, we hypothesized that (i) C substrate addition would enhance soil respiration, and thus denitrification, when D_p/D_o was suboptimal for denitrification, (ii) increasing soil matric potential (reducing D_p/D_o) would increase the rate of denitrification but decrease the N_2O:($N_2O + N_2$) ratio, regardless of C substrate type, and (iii) acetate substrate would enhance N_2O reduction to N_2 relative to glucose regardless of the soil.

2 | MATERIALS AND METHODS

2.1 | Experimental design and site characterization

A few kilograms of soil were collected from three randomly selected locations (0–150 mm depth) in three grazed grassland sites (nine samples in total) all dominated by perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.). The sites were located within a 5-km distance with the same climatic conditions but with different soil types. The soils were collected from the Ashley Dene Research & Development Station (AD, latitude 43° 65′ S, longitude 172° 35′ E, elevation above sea level 34 m, Mottled Argillic Pallic Soil (Hewitt, 1998), Udic Ustochrept (Soil Survey Staff, 1999)), the Lincoln University Dairy Farm (LU, 43° 65′ S,172° 48′ E, Typic Immature Pallic soil (Hewitt, 1998), Typic Haplustept (Soil Survey Staff, 1999)), and the Lincoln University Demonstration Farm (LD, 43° 65′ S,172° 44′ E, Typic Immature Pallic soil (Hewitt, 1998), Typic Haplustept (Soil Survey Staff, 1999)). Soil samples were air-dried and then sieved (≤2 mm), with any visible plant material removed, and stored at 4°C. A subset of each sample was used to characterize soil properties at each site, with three replicates (Table 1). Soil total C and total nitrogen (N) concentrations were analysed on an Elementar Vario-Max CN Elemental Analyser (Elementar GmbH, Hanau, Germany) (Table 1). Total soil phosphorus (P) was determined after sulphuric digestion with concentrated $H_2SO_4 + H_2O_2$ 30% v v^{-1} (Olsen, Sommers, & Page, 1982). Total extractable organic P (Olsen P) was measured after extraction with NaOH 0.25 M+ EDTA 0.05 M (Olsen et al., 1982). Texture analyses were performed using a laser diffraction particle analyser (Mastersizer 3000, Malvern Panalytical, UK). Soil pH was measured on deionized water extracts (Rowell, 2014). The remainder of each sample was used for the experiment. The three samples from each site were amalgamated and then packed into stainless steel rings (73 mm internal diameter, 74 mm depth) to a depth of 41 mm, to achieve a soil bulk density (ρ_b) of 1.1 Mg m^{-3}. The base of each soil core was covered with a fine nylon mesh (25 μm) to prevent any soil loss. The water holding capacity of each soil was determined by immersing the soil cores in water for 2 h and then draining for 24 h (Priha & Smolander, 1999).

For each soil (AD, LU and LD), a factorial experiment consisted of four replicates of two factors: matric potential and C substrate, comprising three levels each of soil matric potential (ψ; −3, −5 and −7 kPa), and C substrate (acetate, glucose, or water as a control). Glucose was selected because it is used commonly as a C source for

TABLE 1 Soil properties for the soils at Ashley Dene Research & Development Station (AD), Lincoln University dairy farm (LU) and Lincoln University demonstration farm (LD)

Site	Soil organic matter (%)	Total carbon (g kg^{-1})	Total nitrogen (g kg^{-1})	Total P (mg kg^{-1})	Olsen P (mg L^{-1})	Clay % (<2 μm)	Silt % (2–63 μm)	Sand % (>63 μm)	Water holding capacity (g H_2O g $soil^{-1}$)	pH
AD	2.3 ± 0.2 b	32.3 ± 0.4 b	3.3 ± 0.0 b	731 ± 25 c	21.3 ± 0.6 c	12	46	42	0.39 ± 0.02	6.2 ± 0.3 a
LU	3.7 ± 0.6 a	46.6 ± 1.0 a	4.5 ± 0.2 a	1,013 ± 1 b	43.7 ± 3.1 ab	16	48	36	0.55 ± 0.01	6.0 ± 0.1 a
LD	3.2 ± 0.5 a	45.3 ± 1.7 a	4.8 ± 0.2 a	1,062 ± 30 a	51.7 ± 6.4 a	17	46	37	0.55 ± 0.01	5.8 ± 0.2 a

Note: Data shown are mean ± standard deviation, $n = 3$. Mean values for each soil were compared using ANOVA and letters represent homogeneous groups obtained from post-hoc analysis (Tukey honest significant difference [HSD] test). Significance levels are given for differences between sites ($p < 0.05$) and means denoted by different letters.

soil organic matter (SOM) priming (Kuzyakov, Friedal, & Stahr, 2000) and to determine C substrate limitation when determining soil denitrification potential (Morley et al., 2014). Acetate, applied as sodium acetate, was selected because it has been shown to increase N_2O reductase efficiency compared to carbohydrates (e.g., glucose) (Morley et al., 2014) and because low-molecular-weight organic acids, such as acetate, occur in the soil due to plant litter degradation, root exudation and organic C decomposition under anaerobic conditions (Castaldelli, Colombani, Vincenzi, & Mastrocicco, 2013). Soil ψ levels were based on those previously observed to give a range of denitrification rates and products (Balaine et al., 2016) and where denitrification rates were observed to be higher between 0 and −6 kPa. In total, 72 soil cores were packed for each soil and this allowed for the destructive analyses of a fully replicated set of treatments on day 3 of the experiment and at the end of the experiment on day 14, which also aligned with the ^{15}N gas emission sampling undertaken on days 3 and 14 as described below.

Soils were maintained at the set soil ψ values by placing the cores on tension tables after they had been saturated with distilled water and allowed to drain for 4 days (Romano, Hopmans, & Dane, 2002). Then 1 mL of a KNO_3, ^{15}N-enriched solution (300 μg N g^{-1} soil or 27.6 mg N mL^{-1}; 40 atom% excess ^{15}N; Cambridge Isotope Laboratories Inc., USA) was applied. The day of KNO_3 addition was defined as day 1 of the experiment. Subsequently, a total of 0.9 mL of C solution was added daily for 14 days (80 μg C g^{-1} soil or 16.4 mg C mL^{-1}) by injecting 0.18 mL of the C soil at five evenly spaced points, to a depth of 20 mm, using a syringe. Tension tables and soil cores were maintained at an average temperature of 20°C.

2.2 | Soil analyses on day 3 and day 14

On day 3 and day 14, pH at the soil surface was measured with a flat surface pH meter (Mettler Toledo, Part No. 51343180) prior to destructive sampling. Soil cores extruded from the stainless-steel rings were homogenized manually and subsampled to determine gravimetric water content (θ_g) by drying at 105°C for 24 h. Soil WFPS was calculated using θ_g, ρ_b and, for all soils, an assumed particle density of 2.65 Mg m^{-3} (Nimmo, 2004). Dissolved organic carbon (DOC) concentrations were determined after extracting homogenized soil subsamples with deionized water for 1 h and then centrifuging (3,500 rpm, 2862 g) the extracts for 20 min before filtering through 0.45-μm cellulose nitrate membrane filters (Ghani, Dexter, & Perrott, 2003). The DOC concentrations were determined on a Shimadzu TOC analyser (Shimadzu Oceania Ltd, Sydney, Australia). Soil inorganic-N was determined by extracting subsamples of the homogenized soil with 2 M KCl for 1 h (1:10 ratio of soil:KCl), centrifuging (3,500 rpm, 2862 g) and filtering (Whatman grade 42 paper). The NO_3^--N and NH_4^+-N concentrations of the KCl extracts were determined using flow injection analysis (Blakemore, Searle, & Daly, 1987).

2.3 | Emissions of N_2O, N_2 and CO_2, and measurement of relative gas diffusivity

Measurements of gaseous emissions were made daily until day 7 and then on days 9, 11 and 14 by placing each soil core into a glass jar (1 L) equipped with a gas-tight lid fitted with a rubber septum. A syringe fitted with a two-way stopcock and a 25G hypodermic needle was used to remove gas samples (10 mL) for measurement of N_2O concentrations, at 30 and 60 min after the jar was sealed. These samples were injected into previously evacuated 6-mL Exetainer® vials (Labco Ltd, High Wycombe, UK) for analysis on a gas chromatograph (SRI-8610, Torrance, CA) equipped with a ^{63}Ni electron capture detector. Increases in N_2O concentration over time (0, 30 and 60 min) were used to calculate rates of N_2O emissions according to Hutchinson & Mosier (1981). Additional gas samples (15 mL) were taken on days 3 and 14, after 180 min, for determination of the ^{15}N enrichment of the N_2O and N_2 evolved using the ^{15}N gas-flux method (Mulvaney & Boast, 1986). These samples were injected into pre-evacuated 12-mL Exetainer® vials. A continuous flow isotope ratio mass spectrometer (CFIRMS, Sercon 20–22; Sercon, Chesire, UK) interfaced to a TGII cryofocusing unit (Sercon, Chesire, UK) was used to measure the ion currents 44, 45 and 46 for N_2O, and 28, 29 and 30 for N_2. Ion currents were subsequently used to determine the N_2O-^{15}N enrichment (Stevens & Laughlin, 1998) and for calculating the N_2 emissions (Mulvaney & Boast, 1986). Standard deviations of repeated measures of ambient air samples ($n = 10$) resulted in $\Delta^{29}R$ and $\Delta^{30}R$ values of 1.4E-6 and 1.1E-6, respectively, and a detection limit of 0.1 mg m^{-2} h^{-1} for N_2. Days 3 and 14 were selected for determining the N_2O and N_2 emissions because, at approximately day 3, Samad et al. (2016a) found that N_2O emissions from pasture soils approached their peak, whereas at day 14 it was expected that soil N_2O emissions would be relatively low because there had been sufficient time for expression and function of N_2O reductase (Liu, Zhang, Bakken, Snipen, & Frostegård, 2019) and utilization of C amendments would have reached steady state.

Soil CO_2 emissions were measured by placing a static chamber on top of the soil core, which was connected to

an automatic soil respiration system (Model LI-8100, Li-Cor Inc., Lincoln, Nebraska, USA).

For both CO_2 and N_2O, daily emissions were calculated and integrated over time to give cumulative emissions over 14 days. In the absence of measurements on days 8, 10, 12 and 13, when soil CO_2 emissions had reached steady state, and soil N_2O emissions had dramatically declined, the Loess model (Cleveland & Devlin, 1988) was used to estimate emissions.

Soil relative gas diffusivity (D_p/D_o) was measured using a gas diffusion chamber (Balaine et al., 2013), which was engineered following Rolston and Moldrup (2002). Briefly, a chamber containing a calibrated oxygen (O_2) sensor (KE-25, Figaro Engineering Inc., Osaka, Japan) was purged with O_2-free air (90% Ar and 10% N_2) while the base of the soil core was isolated from the chamber. Once the chamber O_2 concentration fell to zero, the base of the soil core was exposed to the O_2-free chamber atmosphere and subsequently the elevated O_2 concentration in the chamber, resulting from O_2 diffusing through the soil core into the chamber, was measured after 120 to 180 min. The technique assumes that any error in the calculated value of D_p (O_2 diffusion coefficient in soil) due to O_2 consumption was negligible (Masis-Melendez, de Jonge, Deepagoda, Tuller, & Moldrup, 2015; Moldrup et al., 2000). D_p was calculated from the rate of O_2 increase in the chamber using regression analysis (Rolston & Moldrup, 2002). All diffusivity measurements were made at 20°C and the value of D_o at this temperature was 0.072 $m^2\ h^{-1}$ (Currie, 1960).

2.4 | Data analyses

Differences in the soil properties at the three sites were analysed using ANOVA and are presented in Table 1.

For each soil separately, the effects of the treatments on the temporal evolution of soil CO_2 emissions were tested for significance using a non-linear mixed-effect (NLME) model using the "nlme" package of R (Pinheiro, Bates, DebRoy, Sarkar, & Team, 2017). Each CO_2 emission measurement was treated as a sample, with soil ψ, and substrates set as fixed effect factors. To account for non-independence of repeated measurements over time, the replicate number was included as a random effect in each model. A three-parameter rectangular hyperbola (Crawley, 2007) was fitted to the data as:

$$R_s = a - b\,e^{-ct}, \quad (1)$$

where R_s is the CO_2 emission rate, t is time, a is the value for steady-state CO_2 emissions, b is the difference between the value of CO_2 emissions on a given day and the value of CO_2 emissions on day 0, and the parameter c describes the shape of the curve. Model comparisons were based on Akaike's information criterion (AIC). The model with the lowest AIC indicated the best-fitting model (Anderson & Burnham, 2002) and analyses of residuals were undertaken to ensure that residuals were independent, normally distributed and homoscedastic. Parameter values were compared using Tukey's honest significant difference (HSD) test in the "agricolae" package of R (Mendiburu, 2013).

For each soil separately, the effects of C substrate, soil ψ, and their interactions on soil pH, DOC, NO_3^--N, NH_4^+-N concentrations, the N_2O:($N_2 + N_2O$) ratio, and cumulative values of CO_2-C emissions and N_2O-N emissions were tested using ANOVA in the 'agricolae' package of R version 1.3.1 (Mendiburu, 2013). In addition, cumulative values of CO_2-C emissions and N_2O-N emissions were compared using Tukey's HSD test in the 'agricolae' package of R (Mendiburu, 2013).

3 | RESULTS

3.1 | Effect of the treatments on soil physical and chemical properties

For each soil, the soil pH increased with either acetate or glucose addition compared to the water treatment. On day 3, soil pH values under the acetate treatment (range, 6.3–7.2) were higher than those under glucose (5.9–6.5), which in turn were higher than those for the control (5.4–5.7) ($p < 0.001$; Table S1). Similar findings were observed on day 14, with soil pH values under the acetate, glucose and water treatments ranging from 8.7 to 8.8, 7.1 to 8.3, and 5.3 to 6.0, respectively (Table S2). There was no effect of soil ψ on the soil pH on either day 3 or 14.

As expected, on both days 3 and 14, soil water content was lower in treatments with lower soil ψ. Values of WFPS in the AD, LU and LD soils ranged from 71 to 55%, 90 to 83%, and 94 to 90%, respectively, as soil ψ treatment decreased from −3 to −7 kPa. For the LD soil, WFPS declined as soil ψ decreased from −3 kPa (94%) to −5 kPa (90%) but not from −5 to −7 kPa. When averaged across all soil ψ treatments, soil water content was higher ($p < 0.001$) for the LU and LD soils than for the AD soil. There was no effect of C substrate addition on soil water content.

Values (mean ± standard deviation) of relative soil gas diffusivity (D_p/D_o) in the AD soil were 0.0040 ± 0.0023, 0.0110 ± 0.0019 and 0.0154 ± 0.0028 at −3, −5 and −7 kPa, respectively, whereas for the LU soil the respective values were 0.0026 ± 0.0023, 0.0043 ± 0.0010 and 0.0037 ± 0.0018. In the LD soil the respective D_p/D_o

values at −3, −5 and −7 kPa were 0.0045 ± 0.0024, 0.0048 ± 0.0022 and 0.0058 ± 0.0025. Values of D_p/D_o did not vary with C substrate treatment ($p = 0.817$).

On day 3, DOC concentrations in the acetate (66–254 μg C g^{-1} soil) and glucose (50–254 μg C g^{-1} soil) treatments were higher than those under the control treatment (40–105 μg C g^{-1} soil) in both the AD and LU soils ($p < 0.05$; Table S1). For the LD soil on day 3, the DOC concentrations in the acetate treatment (183–289 μg C g^{-1} soil) were higher than those for the control treatment ($p < 0.05$) but the glucose treatment DOC concentrations were not (138–244 μg C g^{-1} soil; Table S1). On day 14, for all soils, the DOC concentrations in the acetate (180–789 μg C g^{-1} soil) and glucose (68–520 μg C g^{-1} soil) treatments were, when averaged across soil ψ treatments, higher ($p < 0.05$) than those in the control treatment (24–188 μg C g^{-1} soil; Table S2).

On day 3, soil NO_3^--N concentrations were unaffected by treatments, with values ranging from 218 to 361 μg NO_3^--N g^{-1} soil (Table S1). On day 14, in the AD soil NO_3^--N concentrations were lower ($p < 0.05$) at a soil ψ of −3 kPa, in both the acetate (88 μg NO_3^--N g^{-1} soil) and glucose (79 μg NO_3^--N g^{-1} soil) treatments, when compared to the control treatment (242 μg NO_3^--N g^{-1} soil), but this was not the case at −5 and −7 kPa (Table S2). Regardless of soil ψ and substrate treatment the NO_3^--N concentrations, on day 14, in the LU and LD soils (≤60 μg NO_3^--N g^{-1} soil) were consistently an order of magnitude lower ($p < 0.001$) than those in the control (≥130 μg NO_3^--N g^{-1} soil) treatment (Table S2).

Soil NH_4^+-N concentrations did not differ with C substrate on either day 3 or day 14 (Tables S1, S2). At a soil ψ of −3 kPa, the NH_4^+-N concentrations were higher than those at a soil ψ of −7 kPa, with the exception of the AD soil on day 14 where no such effect occurred ($p < 0.05$; Table S2).

3.2 | N_2O and N_2 emissions

For all treatments, N_2O emissions generally peaked between days 3 and 5 (Figure 1). An exception was the less sensitive response to substrate addition, in terms of N_2O emissions, for the AD soil at −7 kPa (Figure 1; Table S3). During this time the N_2O peak emissions were generally highest when glucose was applied ($p < 0.05$; Figure 1). Over the first 7 days, the highest N_2O emissions occurred in the LU soil at −3 kPa when glucose substrate was applied (Figure 1). From day 8, N_2O emissions from the LU and LD control treatments were higher than those from the acetate and glucose treatments ($p < 0.05$; Figure 1). In the AD soil, N_2O emissions were close to zero after day 8, regardless of soil ψ or substrate treatment.

After 14 days, glucose addition resulted in higher cumulative N_2O emissions ($p < 0.05$) when averaged across soil ψ treatments (Table S4). However, soil ψ treatment did not affect cumulative N_2O emissions when averaged across substrate treatments.

On day 3, for all soils, regardless of soil ψ treatment, N_2 emissions were higher following glucose and acetate substrate addition than with water addition, with the exception of the AD soil at −7 kPa where no N_2 flux was measured ($p < 0.05$; Figure 2). N_2 emissions were also higher for the −3 kPa treatment as compared to the −7 kPa treatment ($p < 0.05$), with neither of these treatments differing from the values for the −5 kPa treatment.

On day 14, higher N_2 emissions occurred with acetate than glucose addition in the LU and LD soils ($p < 0.01$) but no such difference occurred in the AD soil ($p = 0.15$). In the LU and LD soils there was no effect of soil ψ on N_2 emissions when averaged across substrate treatment ($p > 0.16$) but N_2 emissions declined ($p < 0.01$) with increasing drainage in the AD soil (Figure 2). The N_2 emissions from glucose-treated LU and LD soils were higher than those from water-treated soils ($p < 0.05$; Figure 2). Averaged across soil ψ potential the ratios of N_2 emissions from the acetate and glucose treatments were 2.56 ± 0.75 (standard deviation), 2.35 ± 0.81 and 0.83 ± 0.31 for the LD, LU and AD soils, respectively.

Carbon substrate type affected the N_2O:($N_2O + N_2$) emission ratio on day 3 (Figure 3), with higher ($p < 0.05$) values under glucose and water (0.91 and 0.90, respectively) than those for soils treated with acetate (0.81). On day 14, the N_2O:($N_2O + N_2$) emission ratios for soils treated with acetate (0.10) or glucose (0.07) were lower than those for the water-treated soil (0.86, $p < 0.05$). The N_2O:($N_2O + N_2$) emission ratio was highest under water-treated LU and LD soils and lowest under glucose-treated LU and LD soils on day 14 ($p < 0.05$; Figure 3). The AD soil N_2O:($N_2O + N_2$) emission ratio did not vary as a result of glucose or acetate treatment at this time. Soil ψ had no effect on the N_2O:($N_2O + N_2$) emission ratio on either day 3 or day 14.

3.3 | Soil CO_2 emissions

Based on the model (Equation 1), the response of CO_2 emissions to glucose or acetate addition was best fitted by an exponential curve (Figure 4). An exception to this was the AD soil treated with acetate at a soil ψ of −3 kPa where the steady state was not reached. Steady-state CO_2 emissions in the other treatments at −3 kPa did not differ with substrate treatment (Table S5). With the exception of the AD soil treated with glucose (2.6 ± 0.2 μmol m^{-2} s^{-1}, $p < 0.05$), where maximum steady-

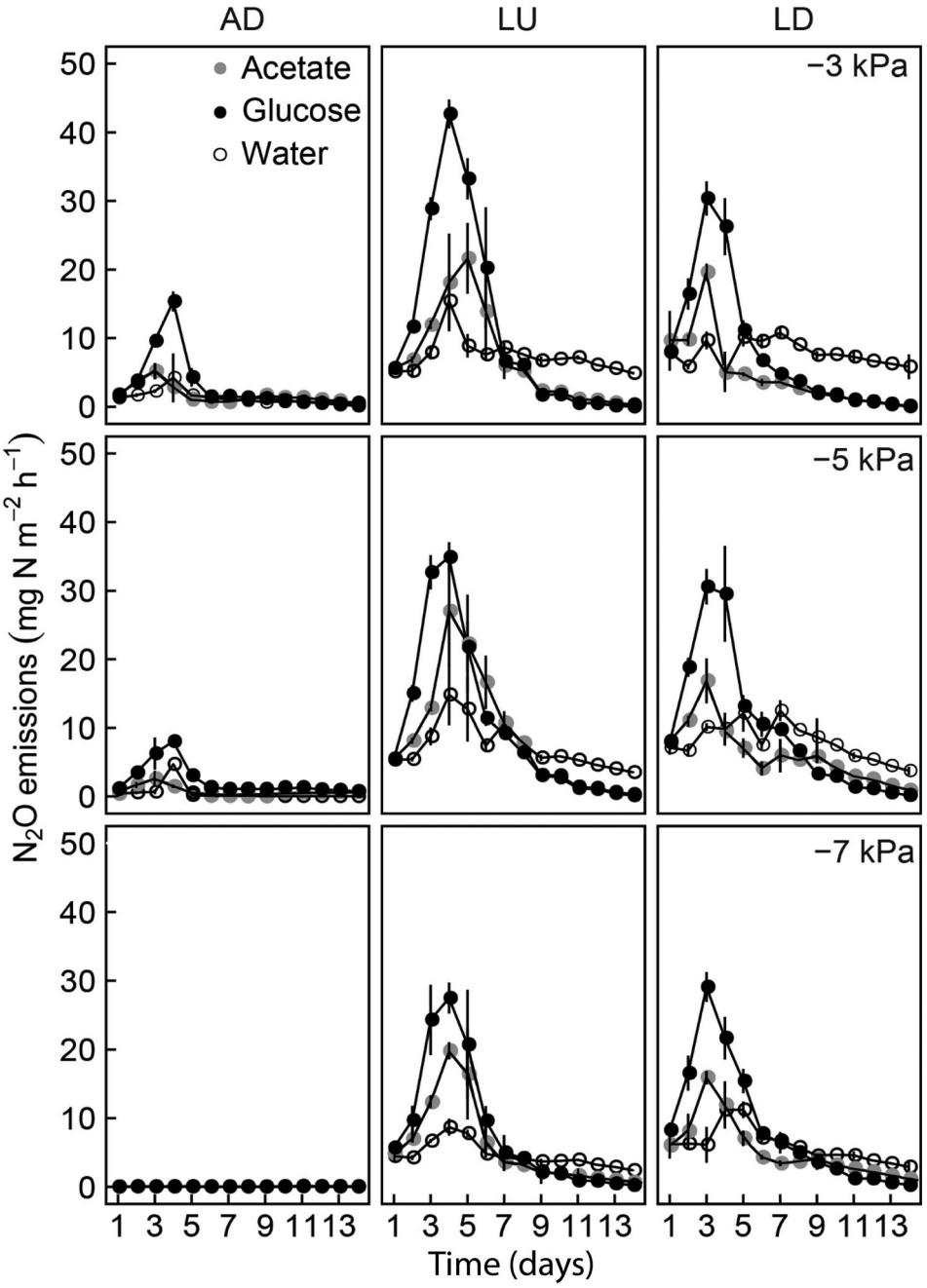

FIGURE 1 Soil nitrous oxide emissions over the 14-day measurement period. Soils were treated with three levels of soil matric potential (−3, −5 and −7 kPa) and three different substrates (acetate, glucose and water). Soils were sampled from three sites: Ashley Dene Research & Development Station (AD), Lincoln University dairy farm (LU) and Lincoln University demonstration farm (LD). Values are means of four replicates (± standard deviation), $n = 4$

state CO_2 emissions occurred at −5 kPa, and the LU soil treated with acetate (1.6 ± 0.0 μmol m^{-2} s^{-1}, $p < 0.05$), where the minimum value of steady-state CO_2 emissions occurred at −5 kPa, there were no differences in the magnitude of steady-state CO_2 emissions at −5 kPa due to substrate (Table S5). Steady-state CO_2 emissions were highest in the AD soil treated with glucose at −7 kPa (Table S5, $p < 0.05$); otherwise, there were no other treatment effects on the magnitude of steady-state CO_2 emissions at −7 kPa.

The rate at which a steady state of CO_2 emissions was reached at −3 kPa generally did not differ with substrate treatment, the exception being the glucose-treated LU soil, which took longer to reach a steady state of CO_2 emissions than with acetate addition ($p < 0.05$; Table S5). At −5 kPa, the glucose-treated AD soils required more time to reach a steady state of CO_2 emissions than the acetate-treated soil ($p < 0.05$; Table S5). There was no difference in the time period required to reach a steady state of CO_2 emissions at −7 kPa as a result of substrate addition (Table S5). There was generally no effect of soil ψ on the time required to reach a steady state of CO_2 emissions in the AD or LU soils. In the LD soil, a higher steady-state value occurred at −7 kPa than at −3 kPa, in both glucose- and acetate-treated LU soil ($p < 0.05$; Table S5).

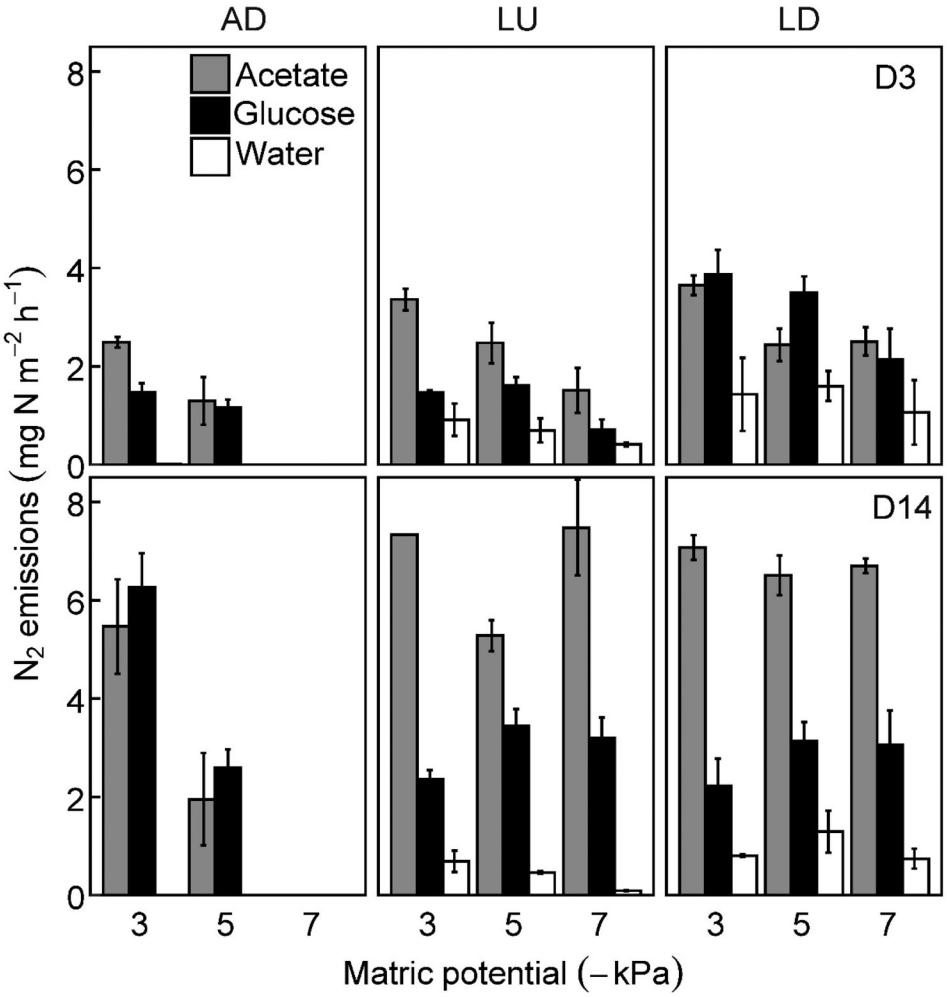

FIGURE 2 The effects of substrate addition and soil matric potential on N_2 emissions on day 3 and day 14 for the three soils at Ashley Dene Research & Development Station (AD), Lincoln University dairy farm (LU) and Lincoln University demonstration farm (LD). Values are means of four replicates (± standard deviation), $n = 4$. The empty spaces represent no detected emissions

For all soils, cumulative CO_2 emissions from the water treatment (control) were lower than those in the acetate and glucose treatments regardless of soil ψ treatments ($p < 0.05$; Table S5). A C substrate by soil ψ treatment interaction resulted in higher ($p < 0.05$) cumulative CO_2 emissions occurring under glucose amendment and as soil ψ became more negative (increasing drainage) (Table S4).

3.4 | Comparisons of CO_2 and N_2O emissions with D_p/D_o

Pooling data showed cumulative N_2O emissions declined exponentially with increasing D_p/D_o, with 67% and 65% of the variation in cumulative N_2O losses explained by glucose and acetate application, respectively (Figure 5). In contrast, pooling the data in a similar manner showed a positive linear relationship between D_p/D_o and cumulative CO_2 emissions, with 47% and 21% of the variation explained by glucose and acetate applications, respectively (Figure 5).

4 | DISCUSSION

Soil WFPS and D_p/D_o showed that soil conditions were suitable for denitrification, with $D_p/D_o < \sim 0.006$ and WFPS > ca. 80% (Balaine et al., 2013; Linn & Doran, 1984; Owens, Clough, Laubach, Hunt, & Venterea, 2017), with the exception of the AD soil, which, due to its higher sand content, held less water at matric potentials of −5 kPa and −7 kPa. Increasing N_2O and N_2 production following application of NO_3^- and C substrates indicates denitrification was the dominant pathway responsible for N_2O and N_2 production. The dominant role of denitrification is also supported by the NO_3^--N concentrations being an order of magnitude lower in the presence of C substrate when soils were anaerobic (D_p/D_o values < ~ 0.006). Dissimilatory nitrate reduction to ammonia (DNRA) can also produce N_2O under anaerobic conditions in grassland soils (Friedl et al., 2018). Higher soil NH_4^+-N concentrations at a soil ψ of −3 kPa than those at −7 kPa suggest only a minor contribution from DNRA given that the soil NH_4^+-N concentrations were relatively low when compared with the

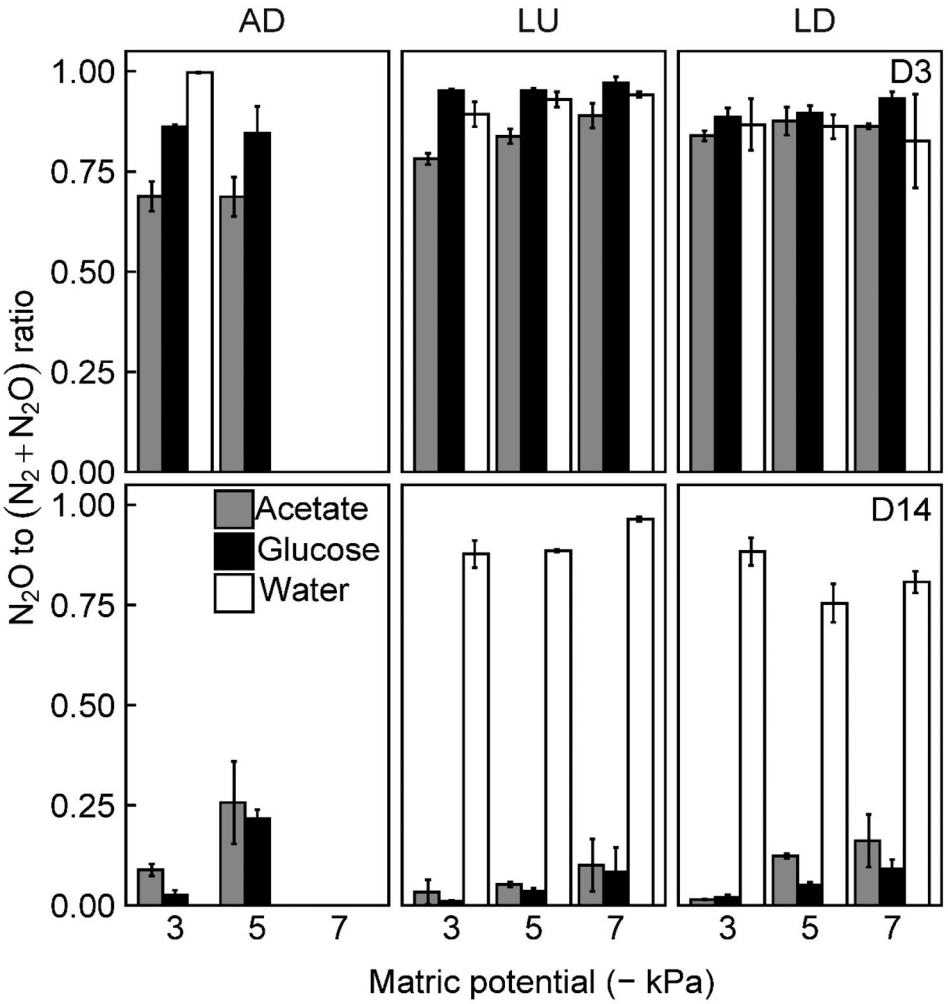

FIGURE 3 The effects of substrate additions and soil matric potential on the ratio of $N_2O/(N_2 + N_2O)$ on day 3 and day 14. Soils were sampled from three sites: Ashley Dene Research & Development Station (AD), Lincoln University dairy farm (LU) and Lincoln University demonstration farm (LD). Values are means of four replications (± standard deviation), $n = 4$. The empty spaces represent no detected emissions

magnitude of the decrease in the soil NO_3^--N concentrations. The low level of NH_4^+ substrate available, a precursor to hydroxylamine, and the low O_2 levels (hypoxic conditions) also imply that anaerobic oxidation of hydroxylamine did not make a significant contribution to the observed N_2O emissions (Stein, 2019).

The low rate of N_2O production in the AD soil (−5 kPa), or lack of both N_2O and N_2 production in the AD soil (−7 kPa), can be attributed to conditions being too aerobic for denitrification ($D_p/D_o > 0.006$), which in turn explains why NO_3^- concentrations remained one or two orders of magnitude higher in the presence of C substrate at −5 and −7 kPa in the AD soil.

Peak N_2O emissions at ~3 days after substrate addition are consistent with the result of Samad, Bakken, et al. (2016a), who examined 13 grassland soils from Ireland and New Zealand that were wetted and amended with NO_3^- before undergoing anaerobic incubation. Upon commencement of the anaerobic incubation, production of NO, N_2O and N_2 occurred, with N_2O production generally peaking at ca. 90 h and N_2 peaking after this time.

Petersen, Schjønning, Thomsen, and Christensen (2008) proposed that increased consumption of O_2, as a result of an enhanced bioavailable C supply, could increase the anoxic zone within a soil. However, the utilization of the applied C substrates in the AD soil as evident from the CO_2 emissions, which were comparable in magnitude to those from the LU and LD soils, was not sufficient to induce anaerobic conditions at −7 kPa in the AD soil based on relative N_2O emissions. Thus, for the data from the AD soil at −7 kPa ($D_p/D_o = 0.0154$), we must reject the hypothesis that enhanced soil respiration following substrate addition will promote denitrification when soil O_2 supply is suboptimal for denitrification ($D_p/D_o > 0.006$). Under these conditions the soil O_2 supply was sufficiently high to maintain aerobic conditions while respiration occurred. However, if we accept (i) the D_p/D_o value of 0.006, shown by Balaine et al. (2013) to demarcate the hypoxic–anaerobic boundary where peak N_2O production and the onset of N_2 production occur (Zhu, Burger, Doaneb, & Howarth, 2013), and (ii) the fact that nitrifying bacteria lack *nosZ* for reducing N_2O to N_2 (Hallin, Philippot, Löffler, Sanford, & Jones, 2018), then the increase in

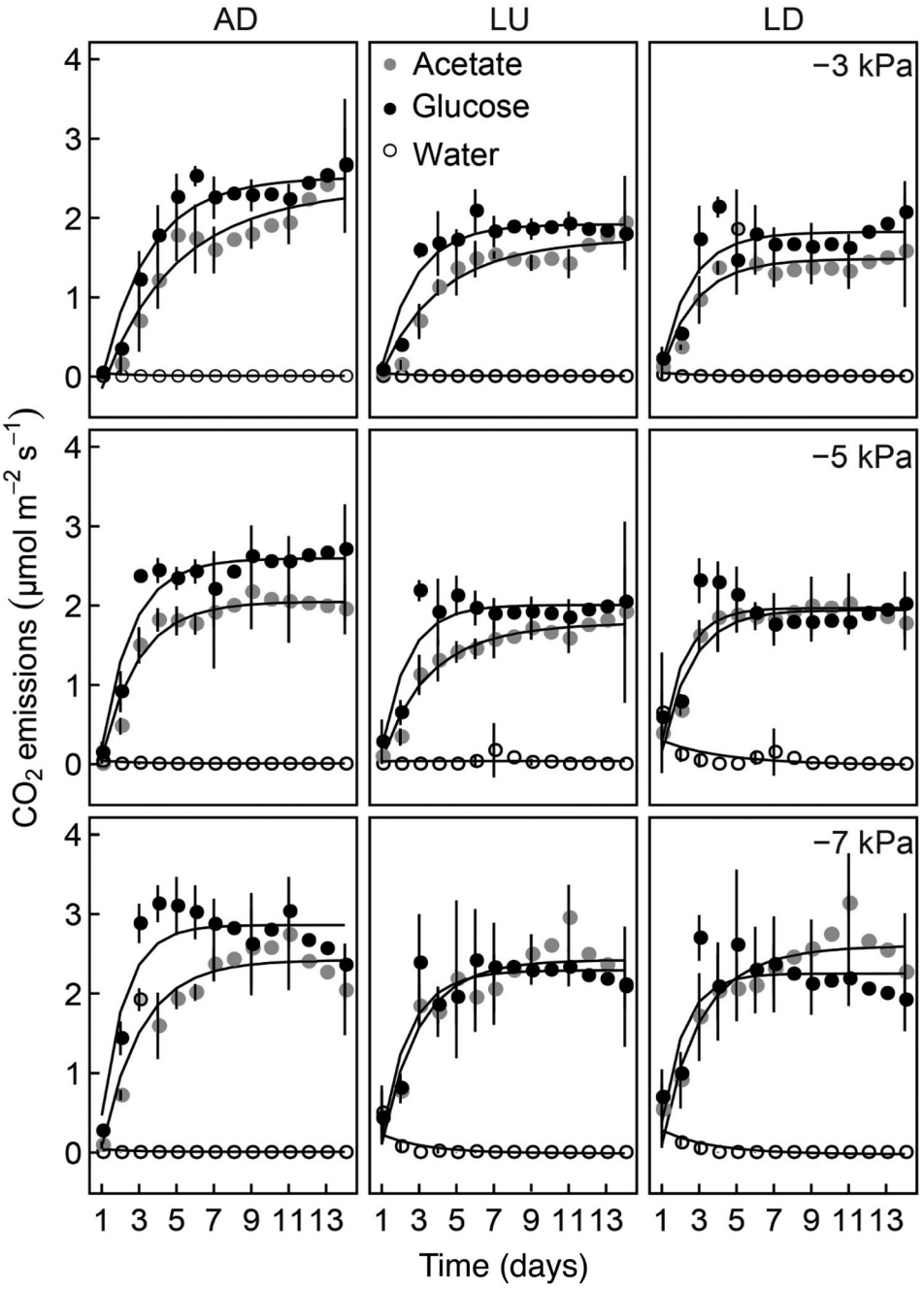

FIGURE 4 CO_2 emissions over the 14 days. Soils were treated with three levels of soil matric potential (−3, −5 and −7 kPa) and three different substrates (acetate, glucose and water). Soils were sampled from three sites: Ashley Dene Research & Development Station (AD), Lincoln University dairy farm (LU) and Lincoln University demonstration farm (LD). Values are means of four replicates (± standard deviation), $n = 4$. Solid lines represent the exponential curve fitted using Equation 1

both N_2O and N_2 emissions in the presence of either of the C substrates, at −5 kPa ($D_p/D_o = 0.0110$), in the AD soil indicates C-induced respiration altered O_2 supply sufficiently to induce anaerobic conditions. Thus, we can accept the hypothesis that enhanced soil respiration following substrate addition will promote denitrification. Similar comparisons cannot be made for the LU and LD soils, where, despite the soil ψ treatments applied, the value of D_p/D_o was constantly ≤0.006, and thus the LU and LD soils were predisposed to denitrifiy on the basis of these anerobic conditions.

Although this current study used repacked soil cores, the results are consistent with an in-situ study on pasture soil that also observed denitrification being promoted when D_p/D_o decreased to ≤0.006 (Owens et al., 2017). Chamindu Deepagoda, Jayarathne, Clough, and Thomas (2019) showed that N_2O fluxes from intact soil cores peaked within a narrow range of D_p/D_o of 0.005–0.010 for soil cores from three soil depths taken from three perennial pastures that received nitrate.

The effect of soil texture on soil O_2 supply is further supported by the observed relationship between cumulative N_2O and diffusivity, where the trend for N_2O emissions to increase exponentially with declining D_p/D_o (ca. < 0.006) aligns with the findings of Balaine

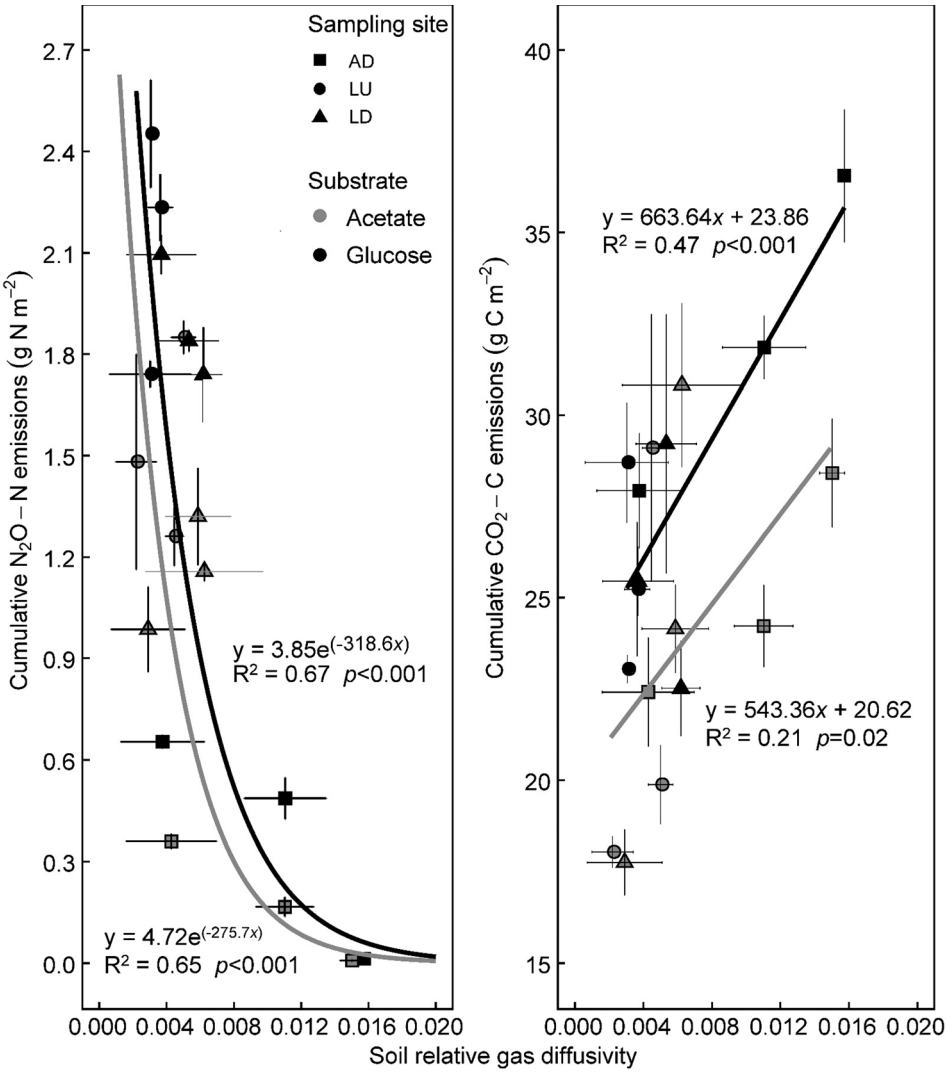

FIGURE 5 The relationship between cumulative N_2O-N emissions, cumulative CO_2-C emissions and soil relative gas diffusivity. Values are means of four replicates (± standard deviation), $n = 4$. Soils from three sites at Ashley Dene Research & Development Station (AD), Lincoln University dairy farm (LU) and Lincoln University demonstration farm (LD) were treated with two substrates (acetate and glucose). Soil matric potentials are not shown as they are incorporated into the calculations of soil relative gas diffusivity

et al. (2013). This was reflected in the absence of (−7 kPa), or relatively lower (−5 kPa), N_2 emissions from the sandy textured AD soil, again likely to be the result of the diffusivity in the AD soil being > ~0.006 (Balaine et al., 2016). Thus, in support of our second hypothesis, declining diffusivity invoked greater rates of denitrification regardless of C substrate type.

We also hypothesized that the ratio of N_2O:(N_2O + N_2) would decrease as the denitrification rate increased but this did not occur. This suggests there were factors other than simply the rate of denitrification influencing this ratio. The rate of denitrification can potentially differ due to microbial community composition, microbial biomass, or the way in which a specific soil's microbial community utilizes an applied C substrate. For example, Giles, Morley, Baggs, and Daniell (2017) found that 120 h after a single input of glucose, glutamine or citric acid, differences in the N_2O and N_2 emissions resulted from differences in C substrate use efficiency. In a study of 13 grassland soils, Samad et al. (2016b) found that the rate of soil denitrification was also closely linked to anoxic C mineralization ($r^2 = 0.89$), measured for 40 h after removal of oxic conditions. Wakelin et al. (2017) found that increasing soil P status increased both microbial biomass and mineralization of added C substrates. Both soil C availability and P status have also been shown to influence soil N cycling (e.g., O'Neill et al., 2021). Hence, the relatively low rate of denitrification observed in the AD soil at −3 kPa may have resulted from both the lower organic matter content and P status of the AD soil generating differences in microbial biomass and microbial community structure that in turn affected how, and at what rate, the applied C substrates were used.

The NO_3^- concentration can also affect the N_2O:(N_2O + N_2) ratio (Conthe et al., 2020). The decline in N_2O emissions by day 14, under glucose and acetate addition, most likely occurred because soil NO_3^- concentrations had also decreased over time. Soil NO_3^- is a preferred electron acceptor to N_2O (Giles, Morley,

Baggs, & Daniell, 2012) and decreasing soil NO_3^- concentrations enables increasing N_2O reductase activity. For example, after applying organic substrates, Senbayram et al. (2012) found that the transformation of N_2O to N_2 occurred more rapidly once soil NO_3-N concentrations decreased below 20 mg kg^{-1} soil. At day 14, this was the case for the LU and LD soils treated with glucose at all matric potentials, and for the LU and LD soils treated with acetate at −3 kPa. Similarly, the increase in soil pH over time will have favoured N_2O reductase activity (Firestone & Davidson, 1989; Samad, Bakken, et al., 2016a). This is because low soil pH (≤6.1) diminishes or prevents reduction of N_2O, primarily by precluding a successful assembly of functional N_2O reductase (Liu, Frostegård, & Bakken, 2014). Consequently, it is also possible that the higher N_2 fluxes observed under acetate could be due partially to the higher soil pH observed under the acetate treatment.

In support of the third hypothesis, on day 3 acetate enhanced N_2O reduction to N_2, relative to glucose, in all three soils (Figure 3), with a lower N_2O:($N_2O + N_2$) ratio observed under acetate (0.81) than glucose (0.91). This effect was not present at day 14 due to the diminished production of N_2O and the dominance of N_2 as a denitrification product as noted above. Previously, Paul et al. (1989) and Morley et al. (2014) showed that the efficiency of N_2O reduction to N_2 was substrate dependent. It has been suggested that acetate is more efficient than glucose in promoting N_2O reduction, possibly due to the differential metabolism of glucose and acetate, with acetate directly entering the tricarboxylic acid (TCA) cycle (Gunina, Dippold, Glaser, & Kuzyakov, 2014), and producing compounds directly linked to the electron transport chain (Conthe et al., 2020; Gottschalk, 1986). However, although the dominance of N_2 production precluded observing the possible effect of acetate on the N_2O:($N_2O + N_2$) ratio at day 14, the more than twofold higher emissions of N_2 under the acetate-treated LU and LD soils, compared with the glucose-treated LU and LD soils, show that, in addition to enhancing N_2O reduction, acetate also increased the overall rate of denitrification at day 14. Gunina et al. (2014) showed that, under non-saturated soil water conditions, similar initial uptakes of glucose and acetate by soil microorganisms occurred after 10 days, but more glucose ^{13}C than acetate ^{13}C was recovered from the extractable microbial biomass, which was interpreted as the result of a higher use efficiency for glucose than acetate. Sugars are metabolized by microbes via glycolysis prior to glucose-C being incorporated into cell components or entering the TCA cycle (Bore, Kuzyakov, & Dippold, 2019) and glucose is recognized as providing the main source of C for a wide range of microbial communities (Paterson, Gebbing, Abel, Sim, & Telfer, 2007), providing more energy than acetate for microbial processes (Paul et al., 1989). However, glucose efficiency as a denitrification C substrate may decline if fermentative bacteria compete with denitrifiers for C (Paul et al., 1989). Given that acetate is generally considered to be a non-fermentable substrate (van den Berg, Elisário, Kuenen, Kleerebezem, & van Loosdrecht, 2017), the lower N_2 emissions observed on day 14 in the LU and LD soils under glucose may have also resulted from greater microbial competition for glucose between fermentative organisms and denitrifiers. However, the fact that the glucose-treated AD soil had similar N_2 emissions to the acetate-treated soil at day 14, suggests that the microbial community in the AD soil was also responding differently to substrate addition with respect to the LU and LD soils due to potential effects of the lower P and C status on the microbial biomass and community structure as noted above. Recent studies have reported increases in N_2O production 2 to 3 weeks after an initial denitrification-induced flux of N_2O is observed, potentially as the result of ensuing mineralization and nitrification (Wu et al., 2017). This may occur depending on soil organic matter content and aeration status. However, observation of such effects was beyond the scope of this experiment.

The fact that the AD soil amended with acetate did not reach steady-state CO_2 emissions at −3 kPa, despite comparable diffusivity with the LU and LD soils at this matric potential, indicates the microbial pool utilizing acetate was still growing, and this is also reflected in relatively low denitrification emissions at −3 kPa in the AD soil at day 3. The lower P status and lower soil C concentration in the AD soil, reflected in the lower DOC concentrations in the control (water only) treatment, may also have resulted in a lower microbial biomass initially being present. The fact that the DOC values were an order of magnitude lower in the glucose-treated AD soil, at −5 and −7 kPa, aligns with the concurrent enhanced diffusivity of these treatments, with an increased oxygen supply driving the CO_2 emissions response in the AD soil in these treatments.

Besides substrate decomposition, CO_2 emissions may also result from substrate-induced priming, stimulating the decomposition of native soil C (Schimel & Weintraub, 2003; Shahbaz, Kumar, Kuzyakove, Börjesson, & Blagodatskaya, 2018). Thus, it is also possible that the observed CO_2 emissions were partly due to priming effects. However, the aim of this study was not to determine priming effects. Future studies are required to examine potential interactions between priming effects and N_2O:($N_2O + N_2$) emissions ratio and gross denitrification rates, as mediated by soil types and C substrate quantity and quality.

The positive response of soil CO_2 emissions to decreasing matric potential that was observed (Figure 5) is in agreement with Groffman and Tiedje (1991), who determined the response of soil CO_2 emissions across the full range of soil water content to be parabolic. For both substrates this positive response was driven strongly by the highest cumulative CO_2 emissions that occurred in the AD soil at the highest diffusivity levels (−5 and −7 kPa), where N_2O and N_2 emissions were relatively low or non-existent.

5 | CONCLUSIONS

By varying soil matric potential to manipulate relative gas diffusivity, emissions of CO_2 and N_2O were measured from three soils amended with NO_3^- and C substrates over 14 days. The results highlight that soil microbial responses to C substrate depend on soil relative gas diffusivity and substrate type. Soil relative gas diffusivity influenced both denitrification and C substrate utilization, with the latter also able to generate anerobic conditions for denitrification by enhancing O_2 demand. Carbon substrate also regulated denitrification products: acetate initially (day 3) produced lower peak N_2O emissions and lower N_2O:($N_2O + N_2$) ratios than glucose. After 14 days, the denitrification emissions were dominated by N_2, with soils higher in organic matter content and with finer texture (lower diffusivity) having twofold greater N_2 emissions under acetate compared with glucose. The time taken to reach steady-state CO_2 emissions, and the maximum rate of CO_2 emissions, varied with C substrate and soil relative gas diffusivity, the latter being a function of soil type.

ACKNOWLEDGEMENTS

This work was funded by the New Zealand Agricultural Greenhouse Gas Research Centre (NZAGRC) and the China Scholarship Council (File no. 201606180104) to support Yuan Li. The authors especially thank Roger Cresswell, Kethsiri Alwis, Emily Huang and Qian Liang, members of the analytical service at Lincoln University, for assisting with laboratory analytical work. We acknowledge also Leanne Hassall, Neil Smith, John Hunt, Graeme Rogers, Carmen Medina Carmona, Marion Delacoux des Roseaux, Adriana Medina Cipagauta, Sephrah Rayner, David Rex and Zach Simpson for their practical assistance.

AUTHOR CONTRIBUTIONS

Yuan Li: Formal analysis; methodology; writing-original draft. **Timothy Clough:** Conceptualization; methodology; resources; supervision; writing-review & editing. **David Whitehead:** Conceptualization; resources; supervision; writing-review & editing.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Li, Y., Clough, T. J., Moinet, G. Y. K., & Whitehead, D. (2021). Emissions of nitrous oxide, dinitrogen and carbon dioxide from three soils amended with carbon substrates under varying soil matric potentials. *European Journal of Soil Science*, 1–15. https://doi.org/10.1111/ejss.13124